IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of : NISHIBAYASHI, et al.

Appln. No. 10/557,747 Group Art Unit: 1612

Filed: November 21, 2005 Examiner: Marcos L. SZNAIDMAN

For: DISINFECTANT AND/OR BACTERICIDAL AQUEOUS COMPOSITIONS

Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. Section 1.132

Sir:

I, Kinue Ohguro, do hereby declare that:

1. I am a Japanese citizen, residing at 422-1 Okinoshima, Kawauchi-cho, Tokushima 771-0142, Japan.

2. I graduated from the Gifu University, Department of Agriculture, in March, 1973.

3. I began my employment with Otsuka Pharmaceutical Co., Ltd. in April, 1982. I have been engaged in new drug research since 1982. Since 1990, I have been engaged in pharmaceutical disinfectant research at the Microbiological Research Institute of Otsuka Pharmaceutical Co., Ltd.

4. I am one of the named inventors of the above-identified application, and am familiar with the subject matter of said application, as well as the disclosures in the cited references.

5. The experiment given below was carried out under my general direction and supervision.

Experimental Data

1. Summary and Purpose of Experiment

A composition containing olanexidine and a polyoxyethylene **alkylphenyl** ether, and a composition containing olanexidine and a polyoxyethylene **phenyl** ether were evaluated for bactericidal effect.

2. Experimental Methods

2-1. Preparation of Compositions

Approximately 85 ml of water was added to 0.1 g of an olanexidine hydrochloride powder (Otsuka Pharmaceutical Co., Ltd.) and 0.32 g of POE (10) nonylphenyl ether (made by Nikko Chemicals Co., Ltd.). Mixing and dissolution were carried out, the pH was adjusted to 5, and then water was further added to give a total volume of 100 mL, thus obtaining a disinfectant and/or bactericidal aqueous composition (sample 1).

Approximately 85 ml of water was added to 0.1 g of an olanexidine hydrochloride powder (Otsuka Pharmaceutical Co., Ltd.) and 0.075 g of POE (10) phenyl ether (made by Aoki Oil Industrial Co., Ltd.). Mixing and dissolution were carried out, the pH was adjusted to 5, and then water was further added to give a total volume of 100 mL, thus obtaining a disinfectant and/or bactericidal aqueous composition (sample 2).

2-2. Antibacterial Activity Tests

Each test strain was cultured overnight at 37°C using a Muller-Hinton broth, successive subcultures were carried out three times, and then the resulting pre-cultured bacterial solution was adjusted to an optical density (OD) at 660nm of 0.3 Abs using sterilized distilled water to make the count approximately 10^8 cfu/mL, and then the solution was further diluted 100 fold with sterilized distilled water to make the count approximately 10^6 cfu/mL, thus obtaining a test bacterial suspension.

Using the test suspension, a 2-fold dilution series was produced using sterilized distilled water such that for each suspension in the series the olanexidine concentration was twise the final test concentration, and 50 μL of each suspension in the series was dispensed into the wells of eight rows in length of a 96-well microplate in order of lowest concentration upward. 50 μL of the test

bacterial suspension was dispensed into each of the wells into which one of the suspension to be tested had been dispensed, and mixing was carried out immediately. 10 μL of each mixed reaction liquid was collected. Five minutes after the disinfecting, the collected reaction liquid was instilled into 200 μL of an SCDLP culture medium (deactivating medium for disinfectant) that had already been dispensed into the wells of another 96-well microplate and mixing was carried out to stop the bactericidal activity, and culturing was then carried out for 48 hours at 37°C.

After culturing, whether or not the bacteria had grown in each well was judged visually from the turbidity of the culture medium with the presence of turbidity being taken as indicating that the bacteria had grown, and the absence of turbidity being taken as indicating that the bacteria had not grown. Out of the dilution series for the test suspension, the minimum concentration for which growth of the bacteria was not observed was taken as the minimum bactericidal concentration (MBC) of the liquid to be tested for the test bacteria.

3. Result

The antibacterial activity test results are shown in Table 1.

Table 1 MBC(μg/mL)

Test Strain	Sample 1	Sample 2
Staphylococcus aureus ATCC 29213	≤ 15.6	250
MRSA 2441-C5606	≤ 15.6	250
Staphylococcus epidermidis ATCC 12228	≤ 15.6	500
Escherichia coli ATCC 8739	≤ 15.6	500
Serratia marcescens IFO 14756	31.25	250
Proteus mirabilis ATCC 4630	≤ 15.6	250
Proteus mirabilis ATCC 27853	≤ 15.6	250

4. Consideration

As shown in Table 1, a composition containing olanexidine and a

polyoxyethylene **nonylphenyl** ether exhibited an MBC less than 1/8 of that of a composition containing olanexidine and a polyoxyethylene **phenyl** ether for all test strains.

As is evident from the results, a composition containing olanexidine and a polyoxyethylene **alkylphenyl** ether has extremely excellent bactericidal effects compared with a composition containing olanexidine and a polyoxyethylene **phenyl** ether.

I, the undersigned, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: January 28, 2010 By: Kinue Ohguro

Kinue Ohguro